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Reduced Rates of Herbicide Metabolism Confer Tri-allate Resistance in *Avena fatua**

Anthony J. Kern,[1] Dwight M. Peterson,[1] Tracey M. Myers,[1] Josette L. Wright,[1] Erica K. Miller,[1] Corey C. Colliver,[1] Maria A. Jasieniuk,[1] Bruce D. Maxwell,[1] Larry L. Jackson[2] and William E. Dyer[1]*

[1] Department of Plant, Soil & Environmental Sciences and [2] Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717-0312 USA

Extensive use of the pre-emergence thiocarbamate herbicide tri-allate [*S*-(2,3,3-trichloroallyl di-isopropylthiocarbamate] over the last 15 to 20 years has selected for resistant (R) wild oat (*Avena fatua* L.) populations in several areas of Montana and Canada.[1,2] Our laboratory's goal was to discover the biochemical mechanism of resistance in order to: (i) better understand the exact mechanism of tri-allate's herbicidal action, (ii) characterize the strategies used by weed plants to escape herbicidal selection pressure, and (iii) generate basic information that may be used to develop field-scale resistance management strategies. R wild oat seeds were collected in August 1993 from fields near Fairfield, Montana in which tri-allate had been used annually for 15 to 22 years[3] from plants surviving treatment the preceeding spring with 1·1 kg ha^{-1} tri-allate. The field collections were shown in greenhouse and Petri dish dose-response experiments to be 6- to 20-fold more tolerant to tri-allate than susceptible (S) lines.[1] One of the R collections was used to develop the inbred R line reported here, through two generations of recurrent selection under 1·1 kg ha^{-1} tri-allate in the greenhouse. R populations and the inbred line were shown to be resistant (8-fold) to the related thiocarbamate herbicide di-allate, as well as to the chemically unrelated post-emergence herbicide difenzoquat (60-fold).[1] S wild oat seeds were collected from field-grown populations of the non-dormant inbred line SH430.[4]

To compare tri-allate uptake and translocation patterns in R and S plants, four-day-old seedlings were treated with [1-^{14}C]tri-allate (1 μl; 1·67 $\times$ 10^3 Bq; sp. act. 3·42 $\times$ 10^6 Bq mg^{-1}) in methanol on the apex of the coleoptile and incubated at 22($\pm$2)°C.[1] After 24, 48, or 60 h, shoots were harvested, washed three times with ethanol + water (90 + 10 by volume; 50 μl) to remove unabsorbed tri-allate, and oxidized in a biological

* To whom correspondence should be addressed: email USSWD@MONTANA.EDU

sample oxidizer. Uptake of ^{14}C was similar in R and S lines up to 24 h after application, with about 4% of the applied herbicide absorbed. However, in contrast to the continued steady uptake in S wild oats, there was no additional uptake of radioactivity in R seedlings between 24 and 60 h after application. Translocation patterns were essentially the same in R and S tissues up to 24 h after application, and most of the label translocated away from coleoptiles was found in underlying shoot tissue. However, more than 70% of the absorbed radioactivity remained in R coleoptiles after 48 and 60 h, as compared to about 50% in S coleoptiles. Thus, decreased tri-allate uptake and translocation in R lines could be partially responsible for the resistance phenotype, although the differences did not appear to be sufficient to confer field levels of resistance.[1]

Tri-allate and other thiocarbamates are thought to require metabolic activation via enzymatic sulfoxidation to exhibit their phytotoxic effects.[5] For this herbicide family, metabolism-based resistance could thus be conferred by a decreased rate of herbicide activation, although this type of resistance has not been previously documented in field- or laboratory-selected plants. To test this possibility, R and S seedlings were treated with [^{14}C]-tri-allate as described above and the metabolites extracted and subjected to HPLC analysis using a C18 reverse phase column in 4·6 × 250 mm format.[6] The mobile phase consisted of water + acetonitrile (95 + 5 by volume) on injection, which was ramped linearly to 50 + 50 by volume over 10 min, ramped to 100% acetonitrile over the next 2 min, and held for an additional 5 min. Labeled compounds were detected using an in-line scintillation counter and peak areas quantified. Tri-allate and the major metabolite, 2,3,3-trichloropropene sulfinic acid (TCPSA), were identified by cochromatography with standards kindly provided by the Monsanto Company. In both R and S plants, tri-allate was metabolized to TCPSA and two minor unidentified metabolites. However, the rate of tri-allate metabolism was more than 12-fold slower in R than in S seedlings (Table 1). Thus, a slower rate of tri-allate activation in R plants could prevent the accumulation of toxic levels of tri-allate sulfoxide, which is thought to be the more herbicidal molecule *in planta*.[5] To test further this idea, we synthesized tri-allate sulfoxide by a modification of the method of Schuphan *et al.*[7] and applied it to R and S seedlings. Both plant types were equally sensitive to tri-allate sulfoxide injury, indicating that the biochemical lesion conferring tri-allate resistance occurs prior to the conversion of tri-allate sulfoxide to additional metabolites. Further, synthetic [^{14}C]-tri-allate sulfoxide was metabolized to the same end-products and at the same rate in both plant types.[6] It thus appears that a deficiency in tri-allate sulfoxidation is responsible for the resistance phenotype in wild oats. Such a deficiency could also explain, at least partially, the reduced uptake and translocation patterns we observed in R plants, since slower removal of tri-allate from R coleoptile and shoot cells would effectively reduce the formation of a tri-allate concentration gradient across the plasmalemma.

To determine whether fatty acid elongases, the proposed target enzymes for tri-allate action,[8] were altered in R plants we treated seven-day-old seedlings with post-emergence applications of tri-allate.[9] Five days after treatment, the seedlings had doubled in size and a clear 'pinching' effect was visible on treated shoots, delineating leaf tissue present before and after treatment. Epicuticular waxes on R and S leaves were isolated by ten 1-s dips in HPLC-grade hexane (0·5 ml), derivatized using bis(trimethylsilyl)trifluoroacetamide, and quantified using GC as described elsewhere.[9] Wax deposition and in-vivo pool sizes of elongated (C > 18) fatty acids were dramatically reduced in treated S but

TABLE 1
[^{14}C]-Tri-allate Metabolism in Susceptible and Tri-allate-Resistant Wild Oats

Time after treatment (h)	*Susceptible*			*Resistant*		
	Tri-allate	*TCPSA*	*Other*	*Tri-allate*	*TCPSA*	*Other*
	radioactivity (% of total recovered)					
1	90	2	8	100	0	0[a]
3	81	8	11	99[a]	0[a]	1[a]
6	63	20	16	98[a]	1[a]	1[a]
9	44	41	15	98[a]	0[a]	1[a]
12	19	61	19	92[a]	2[a]	6[a]
24	7	82	11	87[a]	4[a]	9
36	5	85	11	76[a]	10[a]	13
48	2	80	18	67[a]	22[a]	11
60	1	92	7	69[a]	26[a]	5
72	0	98	2	55[a]	33[a]	12[a]
96	0	91	9	52[a]	43[a]	6

[a] Significant difference from susceptible at $P \leq 0.05$.

not R wild oat leaves. Incorporation of [^{14}C]malonic acid showed that de-novo synthesis of short-chain fatty acids was not affected by tri-allate or tri-allate sulfoxide in R or S plants. In contrast, tri-allate sulfoxide was equally inhibitory to fatty acid biosynthesis and elongation in R and S plants, indicating that elongases in R plants were still sensitive to the activated tri-allate sulfoxide. The results further support the hypothesis that reduced tri-allate sulfoxidation in R plants is the primary mechanism of resistance.

To determine the inheritance patterns of resistance, we made crosses between the inbred R and S lines described above.[10] Seeds from R × S reciprocal crosses and F_2 populations were treated with 0·275, 0·55, and 1·1 kg ha^{-1} tri-allate in the greenhouse, and plant heights recorded after 37 days. Heights of R and S parental plants averaged over treatment rates were 62·5 cm and 0.2 cm, respectively, providing unambiguous classification of phenotypes. Plant heights of F_2 progeny were bimodally distributed with 5·6% and 94·4% of the plants classified as R and S, respectively. Chi-squared tests showed that the observed segregation ratios were consistent with an inheritance model that tri-allate resistance is conferred by two recessive nuclear genes.[10] Inheritance ratios were not affected by treatment rate or parental source of resistance.

Because we have been unable to assay a tri-allate sulfoxidase activity, we do not know if reduced rates of sulfoxidation in R plants are due to lower enzyme levels or to an altered substrate specificity. However, since resistance appears to be controlled by two recessive nuclear genes, we propose that two enzymes are required for tri-allate sulfoxidation, and that mutations in both genes are required for resistance. This report marks the first documented case of herbicide resistance in plants conferred by a reduced rate of metabolism. Further, we know of no other examples of resistance to insecticides, fungicides or antibiotics that are due to a similar lack of metabolic activation. Characterization of tri-allate-resistant wild oats thus represents a novel mechanism of pesticide resistance, and illustrates the diverse strategies by which organisms are able to escape or overcome strong selection pressures in the environment.

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Insecticide Resistance Management in Europe: Recent Developments and Prospects

Ian Denholm[1] & Jørgen B. Jespersen[2]

[1] IARC-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK
[2] Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Lyngby, Denmark

Background

Problems with insecticide resistance in Europe have increased markedly in recent years as a consequence of both the accumulation of resistance mechanisms by some species and the greater diversity of pests attacking valued commodities, especially in the horticultural sector. Arthropods of particular concern include lepidopteran, aphid, whitefly and mite pests of agriculture and horticulture, coleopteran and mite pests of stored grain, and public health and livestock pests including mosquitoes, houseflies and cockroaches. Highly polyphagous thrips and leaf-miners constitute potentially severe problems that are less clearly resolved in a European context. It is also becoming apparent that increased trade in plant material between European countries, and between Europe and overseas, is not only expanding the geographical range of many pests but is also accelerating the spread of resistance genes.[1] The complexity and implications of resistance problems are illustrated well by recent work on two particularly important agricultural pests—the peach-potato aphid, *Myzus persicae* Sulzer, and the cotton or tobacco whitefly, *Bemisia tabaci* Gennadius.

Resistance in Myzus persicae

M. persicae causes direct feeding damage on many crops, but, more importantly, is a major vector of